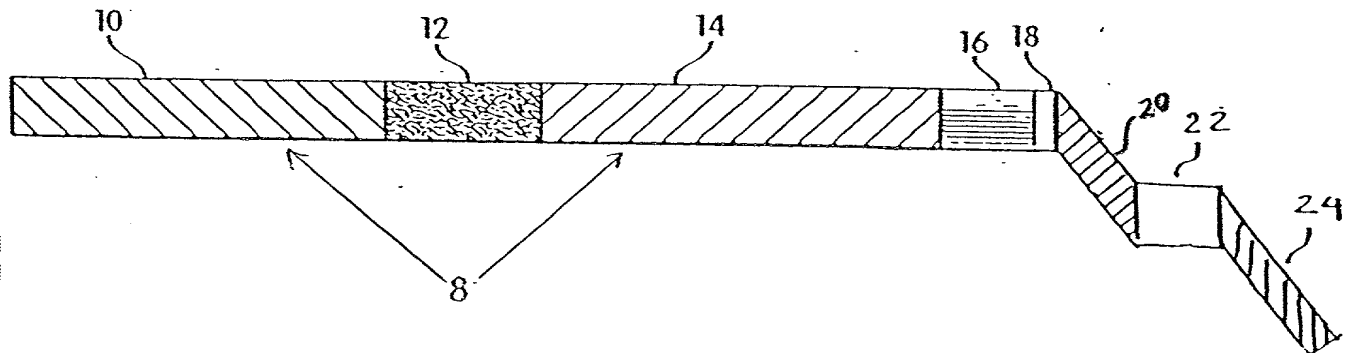
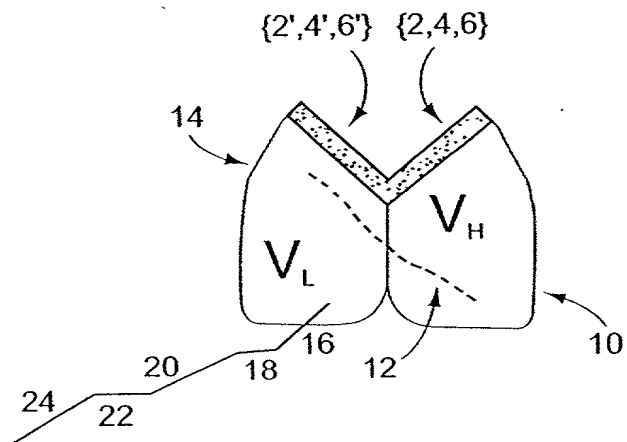


FIGURE 1

SINGLE CHAIN BINDING POLYPEPTIDE



(a) Extended Polypeptide



(b) Folded Protein

FIGURE 2

SINGLE CHAIN
BINDING POLYPEPTIDE SHOWING
LOCATIONS OF COMPLEMENTARITY
DETERMINING REGIONS, POLYPEPTIDE
SPACER REGIONS, AND EFFECTOR REGIONS

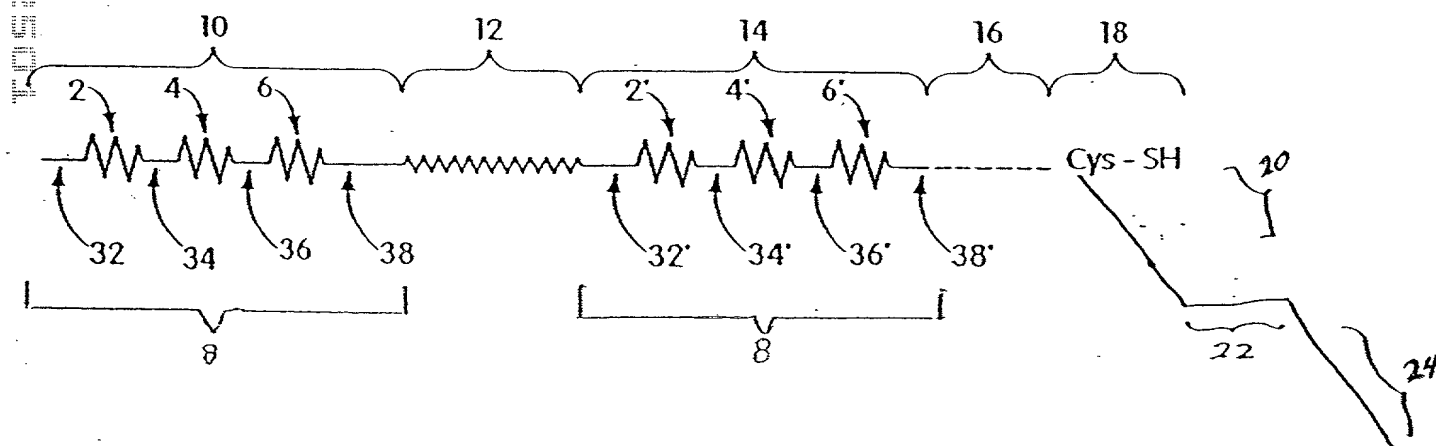


FIGURE 3

C6.5 sFv AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLQLQSGAE LKKGESLKI SCKGSGYSFT SYWIAWVRQM PGKGGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYGHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCAAWDDSL
SGWVFGGGTK LTVLG

FIGURE 4

C6.5 sFv NUCLEOTIDE SEQUENCE

5'caggtgcagctgttgagctctggggcagagtgaaaaaaccggggagctctgaagatctcctgtaagggttctggataca
gctttaccagctactggatcgctgggtgcgccagatgccgggaaaggcctggagtacatggggctcatctatcctggtgactc
tgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtcgcagcactgcctacttgcaatggagc
agtctgaagccctcggacagcgccgtgtattttgtgcgagacatgacgtgggatattgcagtagttccaactgcgcaaagtggcc
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gcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaacagccccaaactcctcatctatggtcacacca
atcggcccgaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtggggtccggtccga
ggatgaggctgattattactgtgcagcatgggatgacagcctgagtggttgggtgttcggcgaggaggaccaagctgaccgtcct
aggt 3'

FIGURE 5

C6ML3-9 sFv' AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLTVVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAH HHHHGGGGC-

FIGURE 6

C6ML3-9 sFv' NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccgaggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcttgggtgcgccagatgcccggaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgctctccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagctctgaagccctcggacagcgccgtgtattttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcgggtggcggatcgcagctctgtgtgacgcagccgccctcagtgctcggccccaggacag
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agcccccaaactcctcatctatgatcaccaatcgcccgaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtggttccgggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgc 3'

FIGURE 7

C6ML3-9sFv'-L1-KDEL AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAH HHHHGGGGCL ESSSSGSEKD EL-

FIGURE 8

C6ML3-9 sFv'-L1-KDEL NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagctctggggcagaggtgaaaaagccccggggagtcctctgaagatctcctgtaagggtctggata
cagctttaccagctactggatcgcttgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgctctccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagctctgaagccctcggacagcggcgtgtatctgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttcagcattggggccagggcacccctggtcaccgtctcctcaggtggaggcggtt
caggcgaggtggctctggcggtggcgatcgagctctgtgtgacgcagccgcccagtgctctgcggccccaggacag
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agccccaaactcctcatctatgatcacaccaatcgcccgaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtggttccgggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcgagggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacgggtggtgg
cggtgcctcagtcct ctagctctgg atccgaaaaa gatgaactg3'

FIGURE 9

C6ML3-9 sFv'-L2-KDEL AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGK¹GLEYMGL
IYPGDSDTKY SPSFQQQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAH HHHHGGGGCL ESSSSGSSSS GSEKDEL-

FIGURE 10

C6ML3-9sFv'-L2-KDEL NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccgaggagtctctgaagatctcctgtaaggggtctggata
cagctttaccagctactggatcgctgggtgcgccagatcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtcctccaaggccaggtcacatctcagtcgacaagtcctgcagcactgcctac
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cctcagcctccctggccatcagtggttccgggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacgggtggtgg
cggctgcctcgagtcta gcagctccgg ttctctagc tctggatccg aaaaagatga actg 3'

FIGURE 11

C6ML3-9 sFv'-L2-H14 AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGGLEYMGL
IYPGDSDTKY SPSFQGGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLTVVSSG GGGSGGGGGSG
GGGSQS SVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAH HHHHGGGGCL ESSSSGSSSS
GSKKSAKKTP KKAKKP-

FIGURE 12

C6ML3-9 sFv' -L2-H14 NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagctctggggcagaggtgaaaaagcccgaggagctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgccctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtcttccaaggccaggtcaccatctcagtcgacaagtcgagcactgcctac
ttgcaatggagcagctcgaagccctcgacagcgcctgtattttgtgcgagacatgacgtgggatattgcagtagttc
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tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgc ctcgagtcta gcagctccgg ttctctagc tctggtacca agaaaagcgc gaaaaagacc ccgaagaaag
cgaagaaacc g 3'

FIGURE 13

C6ML3-9sFv'-L2-nls AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLTVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAH HHHHGGGGCL ESSSSGSSSS
GSTPPKKKRK V

FIGURE 14

C6ML3-9 sFv'-L2-nls NUCLEOTIDE SEQUENCE

5' caggtgeagctggtgcagctctggggcagaggtgaaaaagcccggggagctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcttggtgcgccagatgccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtcctccaaggccaggtcacatctcagtcgacaagtcctcagcactgcctac
ttgcaatggagcagctctgaagccctcggacagcgccgtgtattttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtgccctgaatactccagcattggggccagggcacccctggtcaccgtctcctcaggtggaggcggtt
caggcgagggtggctctggcggtggcggtatcgagctctgtttgacgcagccgccctcagtgctgcggccccaggacag
aaggtcacatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
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tcgggctgggtgttcggcgagggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggtgc ctgagtcta gcagctccgg ttcctctagc tctggatcca ctccgccgaa aaagaaacgt aaagtg 3'

Figure 15. C6ML3-9 sFv' and its salmon protamine conjugate binds specifically to the erbB-2 positive ovarian cancer cells

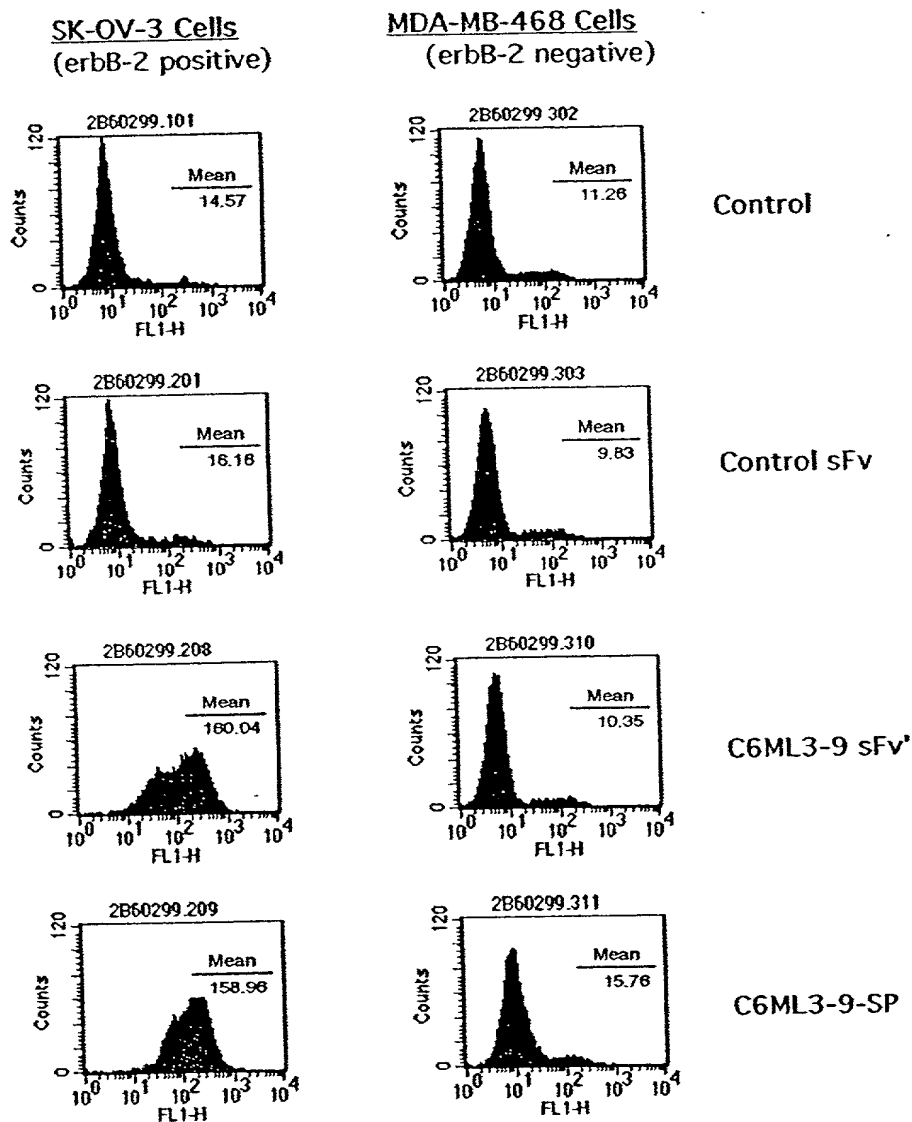
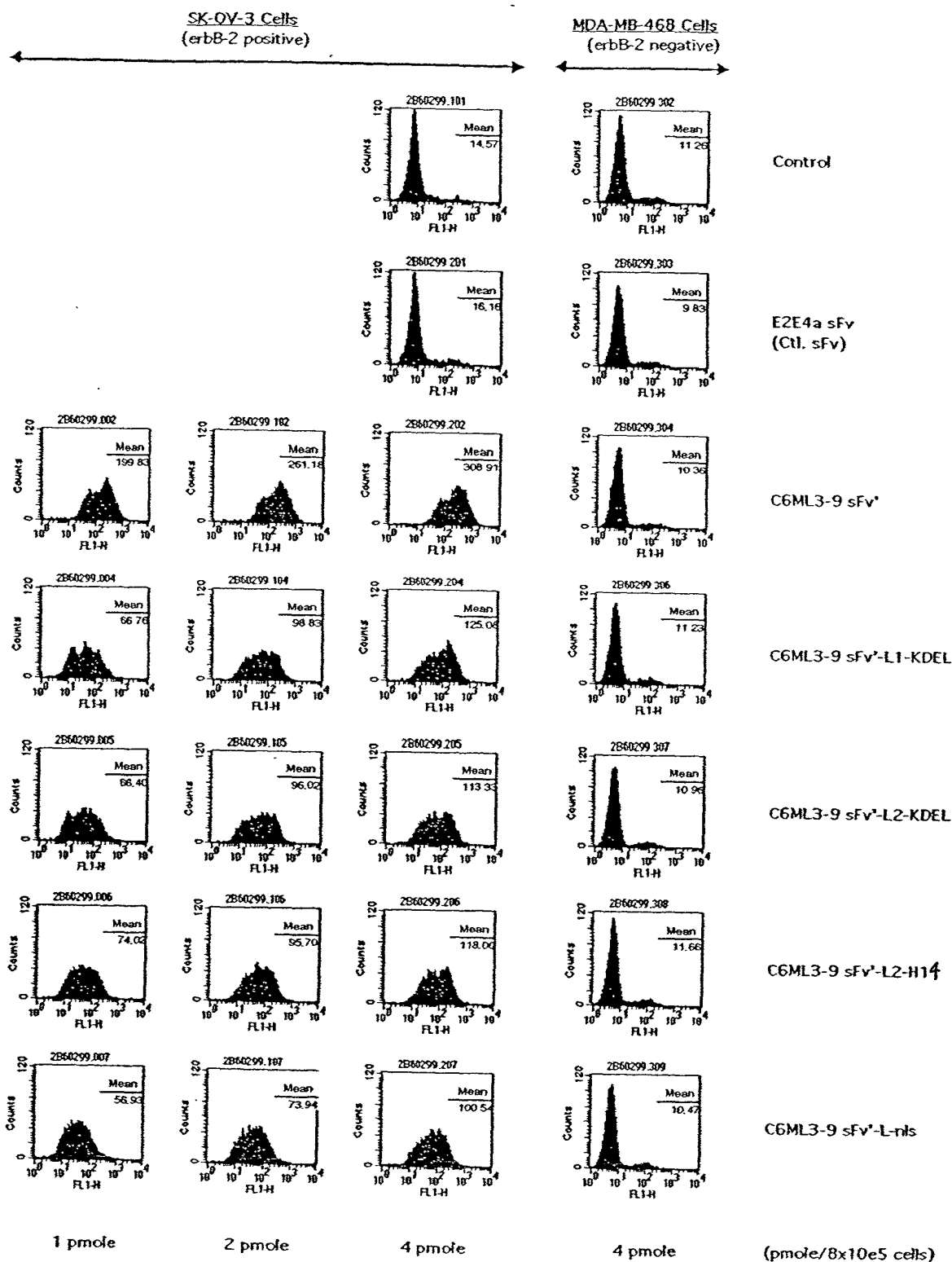
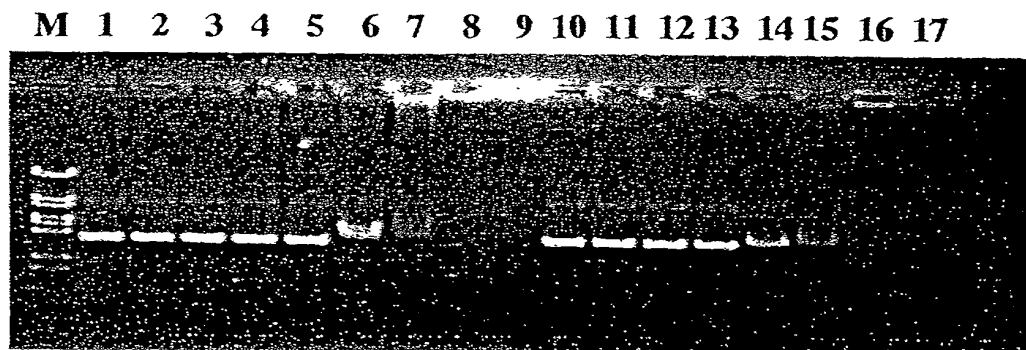


Figure 16. FACS Analysis of the erbB-2 Binding Activities of Bacterially Expressed C6ML3-9 sFv' and its Derivatives



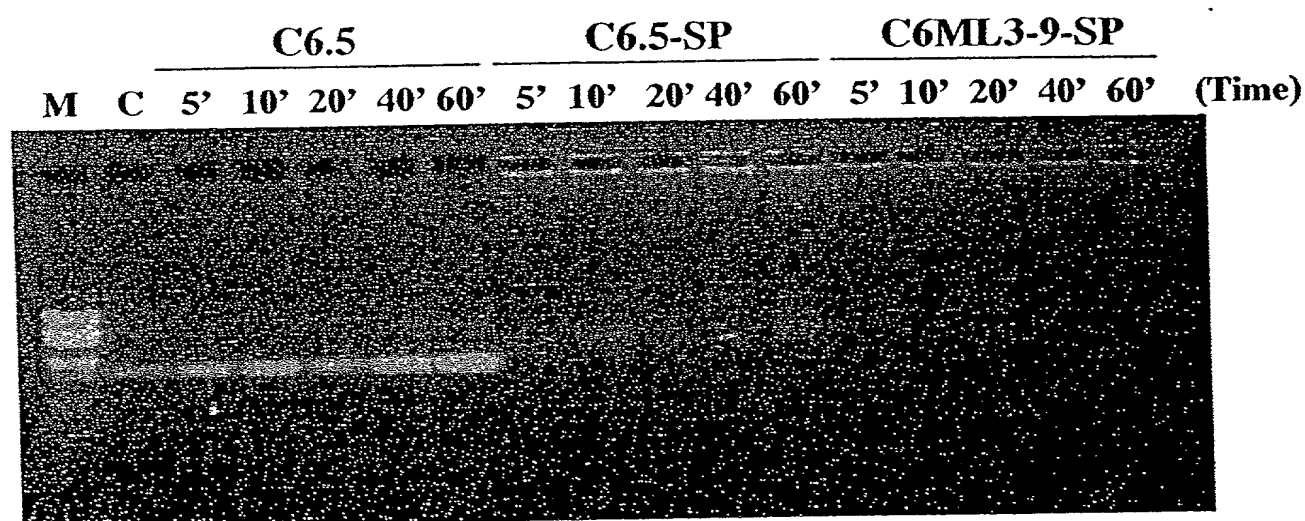
**Figure 17. Gel Shift Analysis of the C6.5-SP-DNA
and C6ML3-9-SP-DNA Complex**



- M. DNA marker - λ DNA BstEII digest
1. 200 ng pGL3 DNA
 2. 200 ng pGL3 DNA + 1.45 pmol C6.5
 3. 200 ng pGL3 DNA + 2.90 pmol C6.5
 4. 200 ng pGL3 DNA + 5.80 pmol C6.5
 5. 200 ng pGL3 DNA + 11.6 pmol C6.5
 6. 200 ng pGL3 DNA + 1.45 pmol C6.5-SP
 7. 200 ng pGL3 DNA + 2.90 pmol C6.5-SP
 8. 200 ng pGL3 DNA + 5.80 pmol C6.5-SP
 9. 200 ng pGL3 DNA + 11.6 pmol C6.5-SP
 10. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9
 11. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9
 12. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9
 13. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9
 14. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9-SP
 15. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9-SP
 16. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9-SP
 17. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9-SP

*0.8% agarose gel in 1xTAE, 150v, RT, ~1hr, EtBr staining overnight

Figure 18. Kinetic Study of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex Formation



M. DNA marker - λ DNA BstEII digest

C. 200 ng pGL3 DNA alone

* The rest of the lanes - 200 ng pGL3 DNA incubated with 5.8 pmol proteins as indicated above each line, on ice, for different period of time. Electrophoresis condition same as Figure 3.

Figure 19. The C6ML3-9-SP conjugate protein mediates specific luciferase gene delivery to erbB-2 positive cancer cells

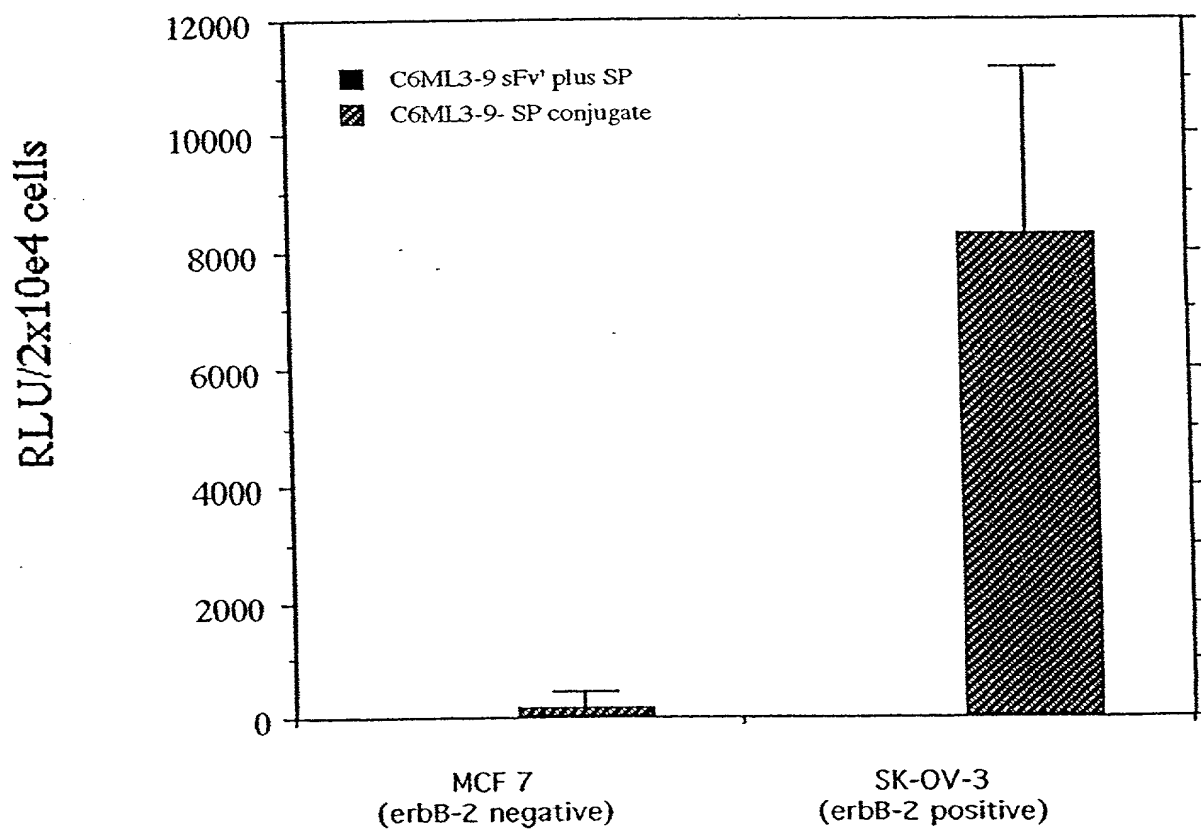


Figure 20. Chloroquine-dependent C6ML3-9-SP-mediated Gene Delivery

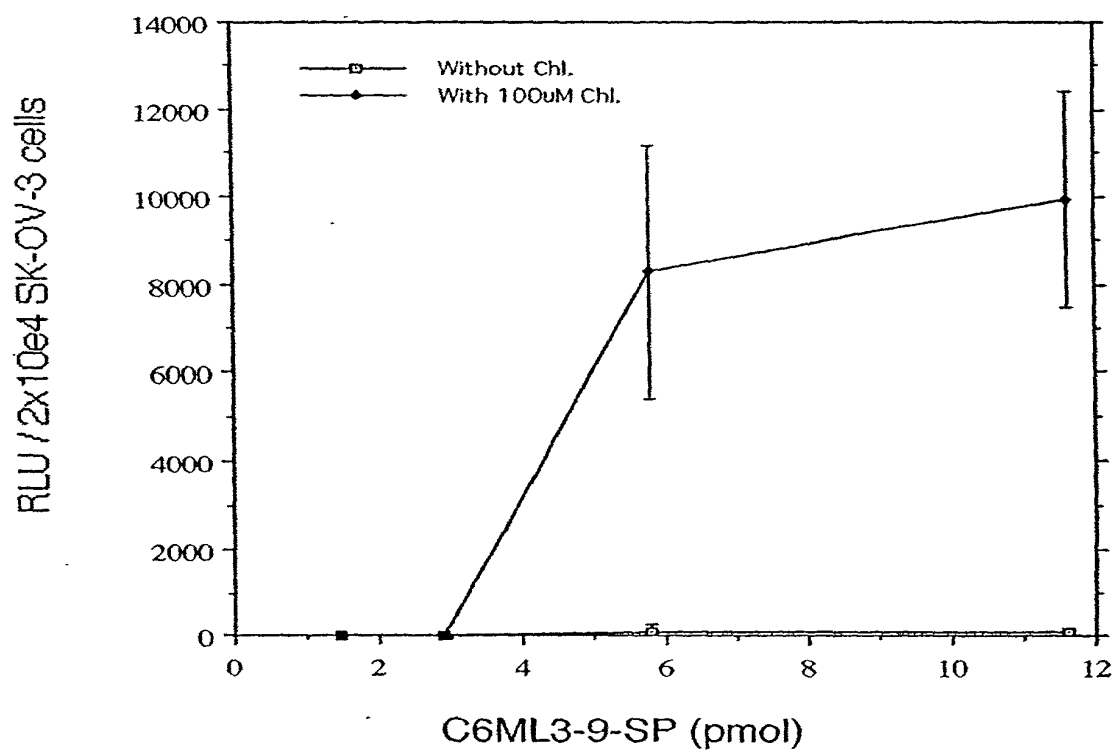
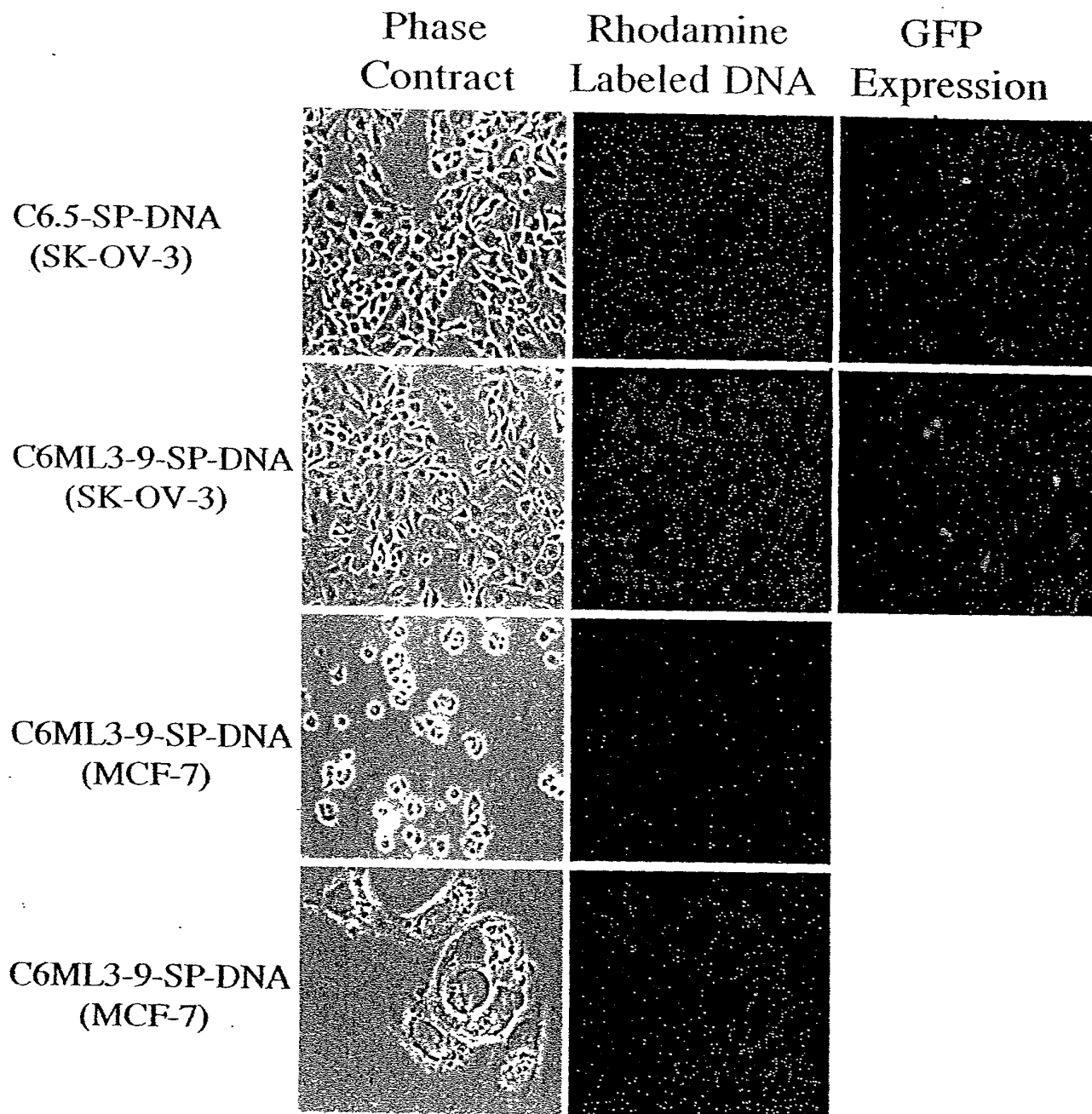


Figure-21. Fluorescent microscopy of C6.5-SP and C6ML3-9-SP-mediated gene transfer of pGeneGrip Rhodamine/GFP plasmids with SK-OV-3 and MCF-7



09083721.062501

FIGURE 22

THE EFFECT OF
CHLOROQUINE ON 3T3-HER2 TRANSFECTION
MEDIATED BY C6ML3-9sFv'-SALMON PROTAMINE

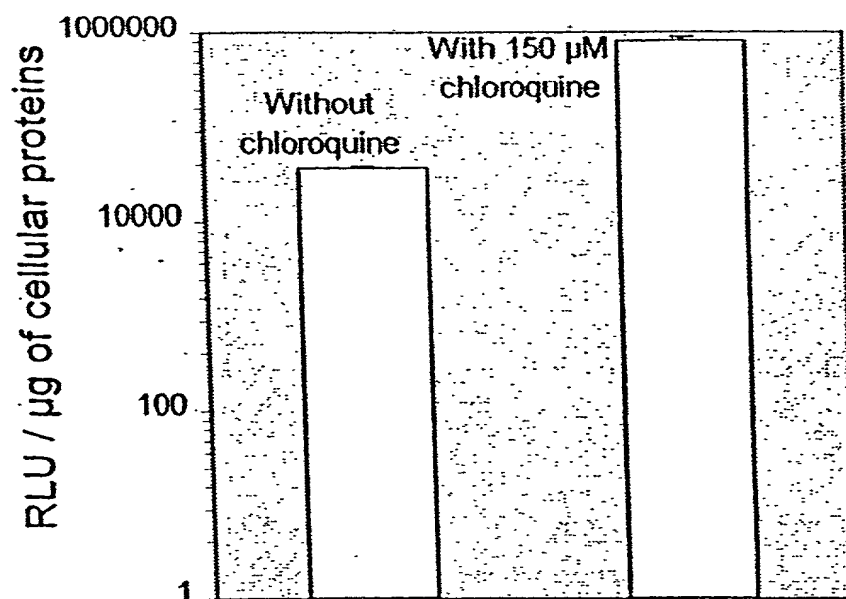


FIGURE 23

THE EFFECT OF CHLOROQUINE ON 3T3-HER2
TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-P1

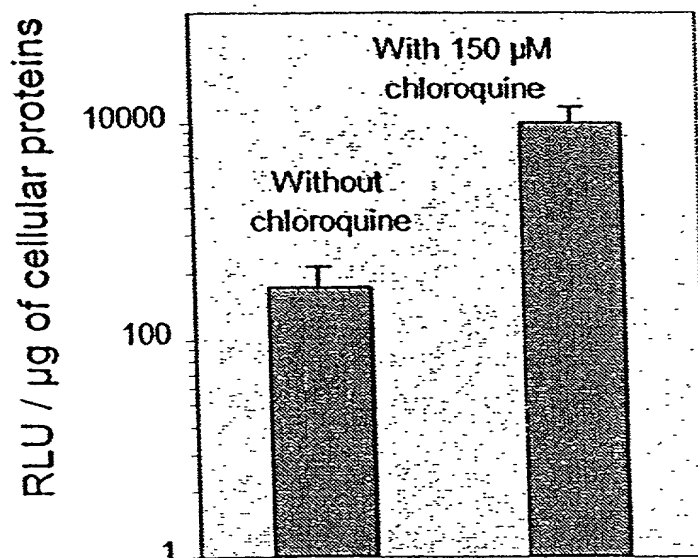


FIGURE 24

THE EFFECT OF CHLOROQUINE ON 3T3-HER2
TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-H1

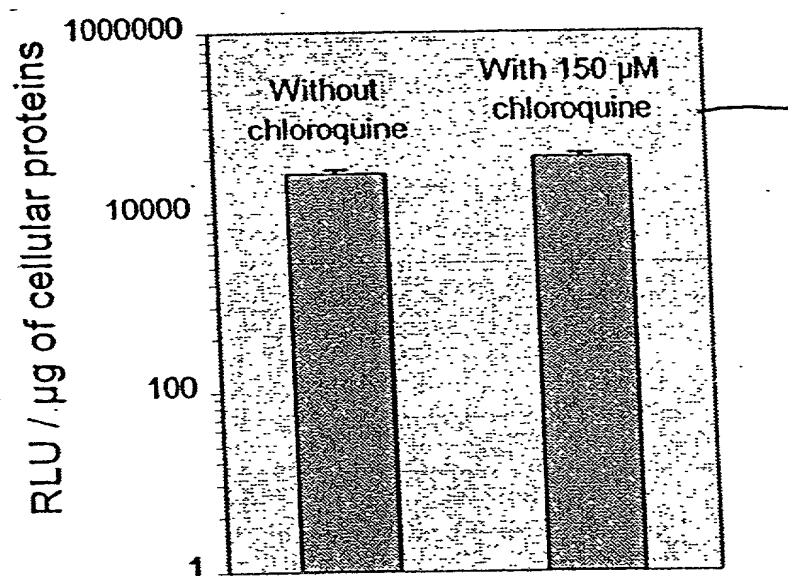


FIGURE 25

THE EFFECT OF
C6ML3-9sFv'-H1-pBks ON 3T3-HER2
TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

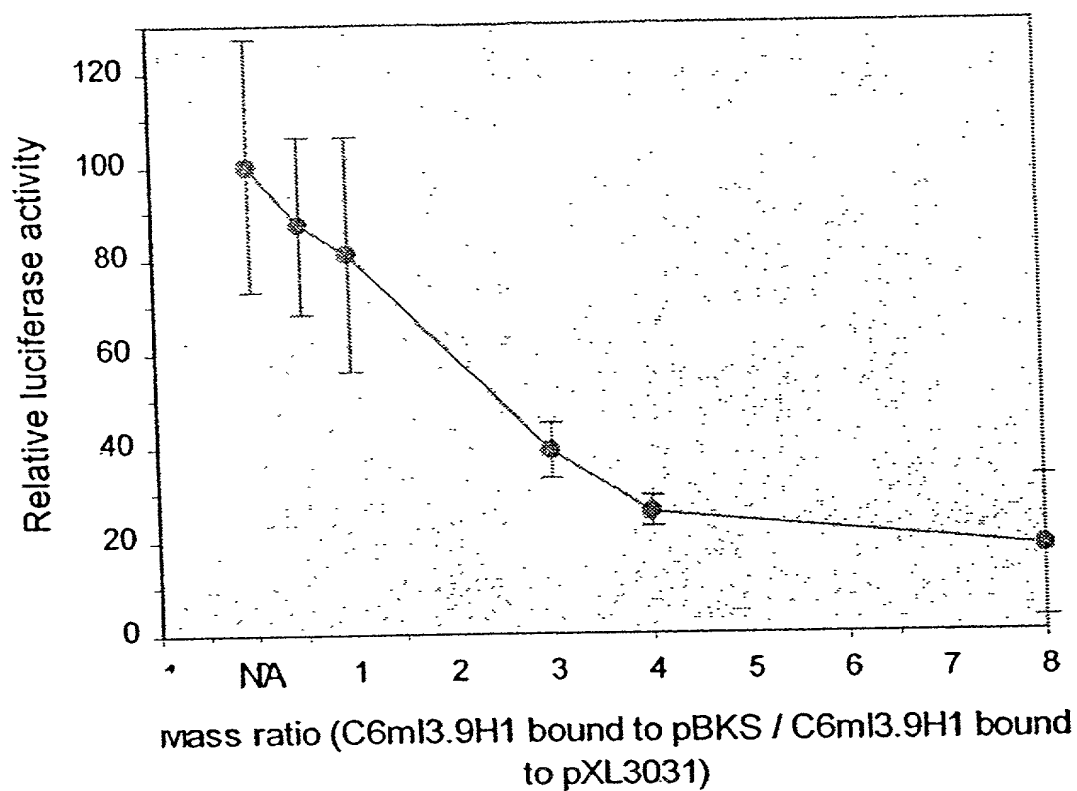


FIGURE 26

THE EFFECT OF THE DNA TO C6ML3-9sFv'-H1
RATIO ON 3T3-HER2 TRANSFECTION EFFICIENCY

